JOURNAL OF LIPOSOME RESEARCH, 2018
https://doi.org/10.1080/08982104.2018.1430829

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Effective suppression of tumour cells by oligoclonal HER2-targeted delivery of liposomal doxorubicin

Alireza Farasat[a], Fatemeh Rahbarizadeh[a], Davoud Ahmadvand[b], Saeed Ranjbar[a] and Shahryar Khoshtinat Nikkhoi[a]

[a]Department of Medical Biotechnology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran; [b]School of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran

ABSTRACT

Synergistic effect of combined antibodies targeting distinct epitopes of a particular tumour antigen has encouraged some clinical trial studies and is now considered as an effective platform for cancer therapy. Providing several advantages over conventional antibodies, variable domain of heavy chain of heavy chain antibodies (V_HH) is now major tools in diagnostic and therapeutic applications. Active targeting of liposomal drugs is a promising strategy, resulting in enhanced binding and improved cytotoxicity of tumour cells. In the present study, we produced four anti-HER2 recombinant VHHs and purified them *via* native and refolding method. ELISA and flow cytometry analysis confirmed almost identical function of VHHs in refolded and native states. Using a mixture of four purified VHHs, PEGylated liposomal doxorubicin was targeted against HER2-overexpressing cells. The drug release was analyzed at pH 7.4, 6.4 and 5.5 and dynamic light-scattering detector and TEM micrograph was applied to characterize the produced nanoparticles. The binding efficiency of these nanoparticles to BT474 and SKBR3 as HER2-positive and MCF10A as HER2-negative cell line was examined by flow cytometry. Our results indicated effective encapsulation of about 94% of the total drug in immunoliposomes. Flow cytometry results verified receptor-specific binding of targeted liposomes to SKBR3 and BT474 cell lines and more efficient binding was observed for liposomes conjugated with oligoclonal VHHs mixture compared with monoclonal VHH-targeted liposomes. Oligoclonal nanoparticles also showed more cytotoxicity compared with non-targeted liposomes against HER2-positive tumour cells. Oligoclonal targeting of liposomes was represented as a promising strategy for the treatment of HER2-overexpressing breast cancers.

ARTICLE HISTORY
Received 11 November 2017
Revised 10 January 2018
Accepted 16 January 2018

KEYWORDS
Active targeting; oligoclonal; VHH; liposome; doxorubicin

Introduction

Despite the extensive success of monoclonal antibody-based treatment in the past two decades, the often modest response to these therapies inspires the development of optimal drug combinations and improved therapeutics. Early attempts of designing novel approaches to target specific antigens, including monoclonal antibody (mAb) combinations (Sym004; bi-clonal), dual-specific antibodies (MEHD7945a) and ADCC-enhanced antibodies (imgatuzumab), have failed to show enhanced clinical activity. As previously shown by Drebin *et al.*, the strategy of combining two or more mAbs against distinct epitopes of the same receptor may improve the efficiency of mAbs (Drebin *et al.* 1988; Kasprzyk *et al.* 1992). Previous studies have attributed this enhanced activity to several factors, which generally lead to increased immune effector cell-mediated cytotoxicity.

Human epidermal growth factor receptor type 2 (HER2) is a transmembrane protein from the ErbB receptor tyrosine kinase family. This family has four members which are collectively referred to as the HER1 (EFGR, ErbB1), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4) (Farasat *et al.* 2017). The expression of HER2 in normal tissues is low and it functions as a vital part of physiological pathways such as survival, differentiation and cell growth regulation (Rubin and Yarden 2001). Overexpression of HER2 has been reported in about 30% of invasive breast cancers (Nuciforo *et al.* 2016), and it also frequently occurs in gastric, non-small cell lung, ovarian and colon carcinomas (Pruszynski *et al.* 2014). Five of the 10 monoclonal antibodies that are FDA-approved for cancer therapy, target either EGFR or HER2. The mAbs, trastuzumab and pertuzumab, and the dual tyrosine kinase inhibitor, lapatinib are some examples of therapeutic agents specifically designed for HER2-positive breast cancer (Wahler and Suh 2015). Monoclonal antibodies are the material of choice in term of specificity for a particular target, but these molecules still suffer from some serious limitations. Herceptin and pertuzumab bind to different sites on the extracellular domain of HER2 (Emde *et al.* 2012). However, patients receiving Herceptin show several side effects, such as the development of drug resistance and cardiotoxicity. Other limitations of mAbs include immunogenicity, large size, difficulty to penetrate dense tissues such as solid tumours and laborious and expensive large-scale production in mammalian cell

CONTACT Fatemeh Rahbarizadeh rahbarif@modares.ac.ir Department of Medical Biotechnology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, P.O. Box: 14115-331, Iran